

# **Characterisation of the Lipidome of Transfusible Platelet Components**

by Sarah Green

Thesis submitted in fulfilment of the requirements for  
the degree of

**Master of Science**

under the supervision of Dr Lacey Johnson and Dr Matthew  
Padula

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Faculty of Science

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## **Certificate of original authorship**

I, Sarah Green, declare that this thesis is submitted in fulfilment of the requirements for the award of Master of Science, in the Faculty of Science, School of Life Sciences at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other institution.

This research is supported by the Australian Government Research Training Program.

30<sup>th</sup> July 2022

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Sarah Green

## **COVID-19 Impact Statement**

The COVID-19 pandemic had a dramatic impact on the progression of my studies and personally impacted me by restricting travel and access to the laboratory to complete my studies. In March 2020, I departed Australia and travelled to the United States of America to visit my husband who lives there. This was intended as a short-term holiday. However, over the next few weeks borders quickly closed and returning to Australia became near impossible. Australian borders were closed for well over 1.5 years and even once they opened, flights were prohibitively expensive and extremely limited. The situation was incredibly uncertain and I prioritised staying with my husband.

Additionally, I was working to obtain my US residency, as upon completion of my studies I was intending to move to the US, and issues arose around this process. It was suggested by our immigration lawyer that I remain in the US until the immigration proceedings could be completed or risk having my US residency application forfeited permanently. Again, I choose to prioritise my husband and my family. Given the border closures and my personal situation if I had returned to Australia, I would have been unable to see my husband for 2 years. It is my belief that being apart from my husband for 2 years would have been extremely detrimental to my own mental health, the mental health of my husband and made progression of my research project extremely difficult.

With uncertainty surrounding border reopening and flights resuming, I took an over 18 month leave of absence, hoping at some point a state of normalcy would resume and I would be able to return to Australia, the laboratory and my studies. However, no such state returned and I was unable to return to Australia. Given this, I could not perform the experiments required to complete an additional results chapter required for a PhD thesis. This study was designed to assess the functional impact for the lipid changes observed in chapters 4 and 5. As such, in May of 2022, I made the decision to complete

data analysis on the data already collected and additional data collected by my supervisors to submit a Masters thesis.

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## **Statement**

This is a traditional thesis, as such it contains an introduction, materials and methods, results, and discussion chapter.

## **Ethics Disclosure**

Ethics approval was obtained for this study from the Australian Red Cross Lifeblood Ethics Committee (Johnson 1107218), and was ratified by the University of Technology Sydney Human Research Ethics Committee (ETH18-2795).

## Publications

### Publications arising from this thesis:

Green SM, Padula MP, Marks DC, Johnson L: The lipid composition of platelets and the impact of storage: An overview. *Transfusion Medicine Reviews* 2020; 34: 108-16.

### Peer reviewed conference abstracts arising from this thesis:

Green SM, Padula MP, Dodgen TM, Batarseh A, Marks DC, Johnson L: Alternative storage of platelets alters the abundance of bioactive lipid mediators. *Transfusion* 2020; 60: 7A-260A.

### Publications alongside this thesis:

Johnson L, Waters L, Green S, Wood B, Marks DC: Freezing expired platelets does not compromise in vitro quality: An opportunity to maximize inventory potential. *Transfusion* 2019; 60: 454-9.

Johnson L, Vekariya S, Wood B, Costa M, Waters L, Green S, Marks DC: The in vitro quality of x-irradiated platelet components in pas-e is equivalent to gamma-irradiated components. *Transfusion* 2021; 61: 3075-80.



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## Abbreviations

ACD	Acid Citrate Dextrose
ADP	Adenosine diphosphate
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BioPAN	Bioinformatics Methodology For Pathway Analysis
C1P	Ceramide 1-phosphate
C1PP	Ceramide 1-phosphate phosphatase
Ca <sup>2+</sup>	Calcium
CCS	Collisional cross section
CDase	Ceramidase
CE	Cholesterol ester
Cer	Ceramide
CerK	Ceramide kinase
CerS	Ceramide synthase
CID	Collision induced dissociation
CLIP-II	Cryopreserved vs Liquid Platelet-II
COX	Cyclooxygenase
CPD	Citrate-phosphate-dextrose
DAG	Diacylglyceride
DIA	Data-independent acquisition
DMSO	Dimethyl sulfoxide
ELISA	Enzyme linked immunosorbent assay
eV	Electronvolts
FDA	Food and Drug Administration
GLA	Gamma-carboxyglutamic acid-rich
GP	Glycoprotein
HDLs	High density lipoproteins
HETE	Hydroxyeicosatetraenoic acid
HLA	Human leukocyte antigens

IMS	Ion Mobility Spectrometry
LC-MS/MS	Liquid chromatography with tandem mass spectrometry
LCAT	Lectin-cholesterol transferase
LDLs	Low density lipoproteins
LOX	Lipoxygenase
LPA	Lysophosphatidic acid
LPC	Lysophosphatidylcholine
LPE	Lysophosphatidylethanolamine
m/z	Mass-to-charge ratio
MAM	Mitochondria-associated membranes
MPV	Mean platelet volume
MS	Mass spectrometry
MS <sup>E</sup>	Mass spectrometry experiment
MTBE	Methyl tert-butyl ether
OCS	Open canalicular system
PA	Phosphatidic acid
PAF	Platelet activating factor
PC	Phosphatidylcholine
PCO	Ether linked phosphatidylcholine
PE	Phosphatidylethanolamine
PEP	Ether linked phosphatidylethanolamine
PF	Pre-freeze
PG	Phosphatidylglycerol
PI	Phosphatidylinositol
PIP	Phosphoinositide
PS	Phosphatidylserine
PSL	Platelet storage lesion
PT0	Post-thaw 0
PT24	Post-thaw 24
PVC	Polyvinylchloride

PVDF	Polyvinylidene fluoride
QToF	Quadrupole Time-of-Flight
RS	Reconstitution solution
RT	Room temperature
S1P	Sphingosine 1-phosphate
S1P lyase	Sphingosine 1-phosphate lyase
S1PP	Sphingosine 1-phosphate phosphatase
SK	Sphingosine kinase
SM	Sphingomyelin
SM D	Sphingomyelin deacylase
SMase	Sphingomyelinase
SMS	Sphingomyelin synthase
sP-selectin	Soluble P-selectin
SSP+	Platelet additive solution
TAG	Triacylglyceride
TF	Tissue factor
TRALI	Transfusion related acute lung injury
TX	Thromboxane
UPLC	Ultra-pressure liquid chromatography
v/v	Volume/volume
vWF	von Willebrand factor

## **Abstract**

Conventional room temperature stored platelets have a short shelf life and require constant agitation. As such, there can be issues when supplying these components to remote and military locations. Alternative storage techniques, such as cold storage and cryopreservation, represent feasible options to circumvent these issues. Cold storage involves storing platelet components in the refrigerator (2-6°C); whereas cryopreservation requires the addition of DMSO and freezing at -80°C. Extensive efforts have been undertaken to understand the changes occurring in cold-stored and cryopreserved platelet components. However, the lipid profile remained incompletely understood. A few historic studies have assessed the lipidome of cold-stored platelets, however, an updated assessment was warranted due to advancements in transfusion practices and mass spectrometry technology. Further, the lipidome of cryopreserved platelets had not yet been characterised. Therefore, the aim of this thesis was to characterise the changes occurring to the lipid profile of alternatively stored platelet components.

The characterisation of the lipid profile of cold-stored and cryopreserved platelet components was conducted as two discrete studies. Apheresis derived platelet components were stored at either room temperature (20-24°C with constant agitation) or cold-stored (2-6°C without agitation) and sampled on day 1, 5, and 14 post-collection. Buffy coat derived platelet components were frozen with 5-6% DMSO and stored at -80°C. Frozen components were thawed and then stored at room temperature for 24 hours, and samples were taken before freezing, after thawing and after post-thaw storage. The platelet, microparticle and supernatant fractions were separated by differential centrifugation. Lipids were extracted using methyl tert-butyl ether (MTBE) and the lipid profile of the component fractions were assessed by LC-MS/MS. Several bioactive lipid mediators were assessed by ELISA.

The lipid profile of platelets was relatively unchanged during storage for 5 days, regardless of temperature. However, over extended storage (14 days)

changes became apparent, and these were exaggerated by cold storage. Conversely, the lipid profile of the supernatant was changed during early storage at both temperatures, but changes stabilised during extended storage. These changes may be the result of an exchange of lipids between the fractions. More specifically, the proportion of the procoagulant lipids, PS and PE, increased during extended cold storage. Further, several LPC species associated with inflammation were altered during extended room temperature storage. Most interestingly, alterations were observed in apoptosis-associated ceramide species suggesting that cold storage of platelets may delay the progression of apoptosis.

The lipidome of the cryopreserved platelets was not considerably altered immediately after thawing. However, changes became apparent during post-thaw storage. In contrast, the lipid profile of microparticles formed after thawing was significantly different to the lipid profile of the microparticles present prior to freezing. The changes present after thawing are likely the result of interactions between fractions. More specifically, externalisation of lipids associated with coagulation (PS and PE) were increased immediately after thawing. Further, lipid changes present in the post-thaw microparticles and the supernatant (LPC and LPE) may be associated with altered inflammation and signalling.

Overall, the research presented in this dissertation has expanded the knowledge of alternatively stored platelet products and thus may be valuable in expanding their utility.